UNIVERSITAT DE BARCELONA

MASTER FINAL PROJECT
MASTER OF ENVIRONMENTAL ENGINEERING

Abatement of Clothianidin pesticide by Ozonation

Author

Lucas Cruz Diego

21/06/2019

Director/s

Dr. Carme Sans Mazón

Department of Chemical Engineering and Analytical Chemistry. University of Barcelona

Dr. Alberto Cruz Alcalde

Department of Chemical Engineering and Analytical Chemistry. University of Barcelona

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1. INTRODUCTION

Water is a limited natural resource, frequently deteriorated due to continuous development and industrialization. The manufacture of chemical products has been increasing and with it, the number of compounds considered potentially dangerous for the environment and human health that can be found in wastewaters.

In recent years, the emerging contaminants (ECs) have aroused considerable interest. They are considered emerging because they are not yet regulated or are being subject to a regulatory process [1].

These compounds are included in the formulations of personal care products, plasticizers, pharmaceuticals, pesticides, surfactants, etc. and removing them from these products seem to be difficult, at least in short-term. However, most of the researchers are of firm opinion that legislative intervention by governments can help to control this contamination. At present, the use of these chemicals and their resistance to conventional water and wastewater treatments has caused their detection at low concentrations in water.

ECs are not regulated around the world but there are some attempts in EU and North America to make a priority list and reduce their release into the environment. For example, in Canada and Switzerland, several projects were performed to develop strategies for reduction of ECs release improving wastewater treatment plants (WWTPs). [2]

In the last 10 years, a rather fast evolution of the research activities devoted to environment protection has been recorded as a consequence of the special attention

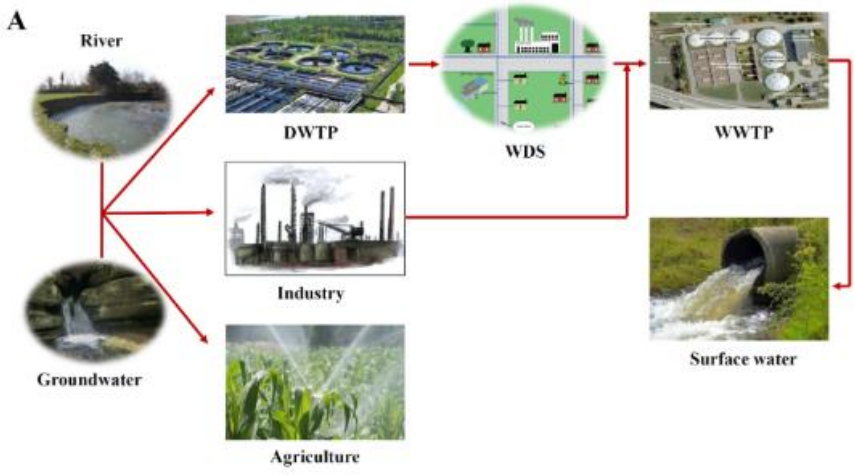

Figure 1. Linear strategy for water. [3]

paid to the environment by international authorities. As well as other conventional manufacturing lines, our current drinking water and wastewater treatment plants (Figure. 1) follow the old linear strategy in which there is a “lifetime” for the products . [3]

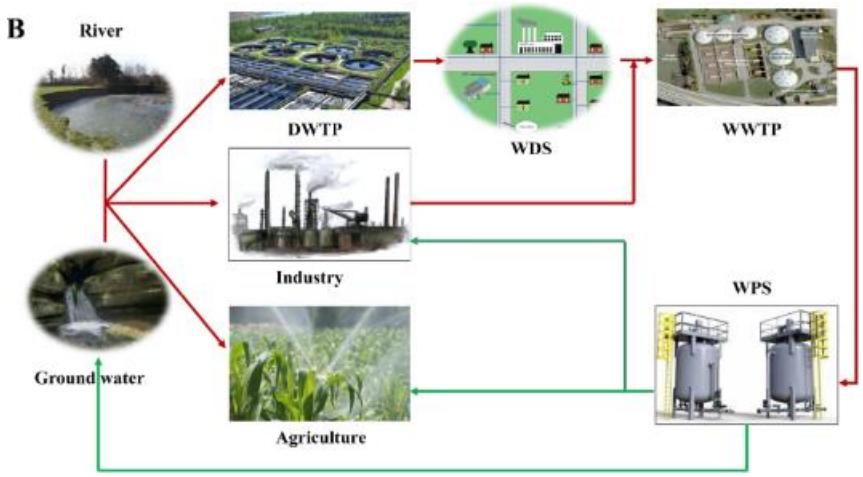

Figure 2. Circular strategy for water. [3]

A switch to the circular strategy, in which the products are completely recycled and the processes with least waste production are preferred, has been successfully implemented for many metallic and plastic products. This attitude should be extended to water distribution and municipal wastewater collection systems which currently release different pollutants including contaminants of emerging concern along with the wastewater effluent into the environment. [2].Consequently, nowadays the researchers are looking for some alternatives to reduce these micropollutants present in the waters. One of the most important mitigation strategies is the use of Advanced Oxidation Processes (AOP's).

1.1 ADVANCED OXIDATION PROCESSES

Advanced oxidation processes (AOPs), defined as those technologies that use hydroxyl radicals (·OH) for oxidation, have received increasing attention in the research and development of wastewater treatment technologies in the last decades. These processes have been applied successfully for the removal or degradation of toxic pollutants or used as pretreatment to convert recalcitrant pollutants into biodegradable compounds that can then be treated by conventional biological methods. The efficacy

of AOPs depends on the generation of reactive free radicals, the most important of which is the hydroxyl radical (·OH). [4]

There are different types of advanced oxidation processes, including Heterogeneous Photocatalysis, H_2O_2-UV, Fenton-based reactions, and ozone-based processes.

Chemical oxidation aims at the mineralization of the contaminants to carbon dioxide, water and inorganic compounds or, at least, at their transformation into harmless products.

One of the most important advantages of water treatments based on chemical destruction, such as AOPs, is that when properly developed, they give a complete solution to the problem of pollutant abatement. This is in contrast with those processes in which only a phase separation is realized with the consequent problem of the final disposal. [5][6]

1.1.1 HYDROXYL RADICAL

The hydroxyl radical is characterized by having the second highest oxidation potential, after fluorine (Table 1).

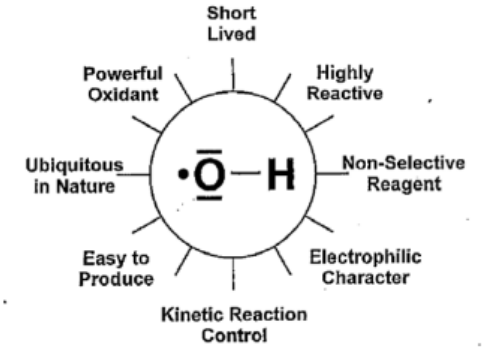

Figure 3. Characteristic of Hydroxyl Radical. [7]

Table 1. Oxidation Potential. [7]

Oxidant	E° (V)
Fluorine	3.03
Hydroxyl radical	2.80
Atomic oxygen	2.42*
Ozone	2.07
Hydrogen peroxide	1.77
Potassium permanganate	1.67
Hypobromous acid	1.59
Chlorine dioxide	1.50*
Hypochlorous acid	1.49
Chlorine	1.36
Oxygen	1.20*
Bromine	1.09

The hydroxyl radical, in addition to be an extremely powerful oxidizing agent, has among other features: short life, non-selective, high reactivity, easy to produce, moreover other characteristics included in figure 3. [7] Because of , the hydroxyl radical has the characteristics before mentioned, the removal of this micropollutants (MPs) can be carry out at low concentrations.

Hydroxyl radicals formed by AOPs react by hydrogen abstraction, electrophilic addition or electron transfer reaction.

Photolysis-UV

Many studies have demonstrated the possibility of degrading pollutants using direct UV photolysis, which improve the treatment of water without the need to add chemical reagents. It must be considered that, for example, a photon of 254 nm is equivalent to 4.87eV, enough energy to produce homolytic and heterolytic breaks in molecules.

Direct irradiation leads to the promotion of a molecule from the ground state to an excited state, which can make intersystem crossings to produce triplet states. In most cases, homolytic rupture produces radicals.

It has been observed that the degradations vary greatly depending on whether they are applied to the compounds separately or in a mixture. It has also been observed that the degradation depends on the UV absorption of each compound.

UV-H_2O_2

This process is affected by irradiating the pollutant solution containing H_2O_2 with UV light with a wavelength smaller than 280 nm. This causes the homolytic cleavage of H_2O_2.

$$H_2O_2 + h.\nu \rightarrow 2 \bullet OH$$

Some characteristics of this method are:

- Oxidation rate compared with single photolysis increased by the presence of radicals
- Produce these radicals at lower operating costs.

- Low and medium pressure mercury arc lamps are used
- The overall quantum yield is close to one ($\phi_{H2O2} = 0{,}98\ to\ 254nm$).

The major drawback of this process is the small molar extinction coefficient of H_2O_2 which is only 18.6 M^{-1} cm^{-1} at 254 nm. Only a relative small fraction of the incident light is therefore exploited, in particular in waters where organic substrates will act as inner filters.

The rate of water photolysis of H_2O_2 has been found to be pH dependent and to increase when more alkaline conditions are used. This may be primarily due to the higher molar absorption coefficient of the peroxide anion $HO2^-$ which at 254 nm is 240 M^{-1} cm^{-1}.

1.1.2 OZONE

Ozone, considered as a homogeneous type of AOP, is the fourth most powerful oxidant, exceeded in its oxidation potential only by fluorine, hydroxyl radical and atomic oxygen. The chemistry of ozonation is complex and is characterized by acting through two mechanisms: direct reaction, with dissolved molecular ozone and indirect reaction, with hydroxyl radical formed when ozone decomposes in water combination of these two mechanisms in the removal of chemical substances, mostly depends on the water properties and the applied dose of ozone.[8]

Ozone is 14 times more soluble in water than oxygen. The solubility decreases with temperature and increases with pressure and it must be generated in situ. There are three methods to generate the ozone from oxygen: UV (185 nm) irradiation, Electrolytic generation and Electric discharge. The last is the one which we used in this work.[9]

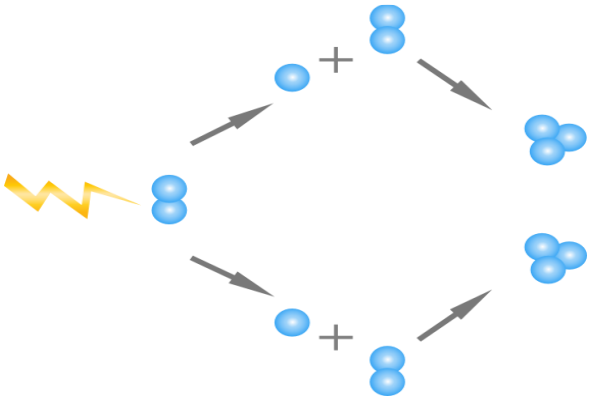

Figure 4. Ozone generation by Electric Discharge. [9]

Ozone is obtained by passing a current of air or oxygen between two electrodes subjected to a high alternating potential.

The system is increasingly used to decompose the oxygen molecule (O2) of the air in two oxygen atoms (O1), which join another oxygen molecule (O2) to form the Ozone (O3), which can be then used for water treatment.

The corona effect is due to the accumulation of high potential electric charges in the conductors. When this accumulation of electric charges reaches saturation, the surrounding air becomes slightly conductive and electrical charges escape, producing a characteristic sound and emitting light. [10]

1.2 CLOTHIANIDIN. COMPOUND PROPERTIES AND USE

Pesticides are organic compounds of an anthropogenic nature, which represent a high risk to the health of people, fauna, and the environment. Currently, about 16 million different pesticides are known and approximately 250,000 new compounds are synthesized every year.

Although pesticides were regulated decades ago, the problem lies in their degradation products, most of them are polar, practically ignored until now, but which can be more toxic even than the starting products. [1]

Nevertheless, many classes of insecticides have been detected recently in wastewaters, after their use and the absorption on the superficial water and underground water, due to incomplete removal in wastewater treatment plants (WWTPs), affecting water quality. [11]

Clothianidin (CLO) is a second-generation neonicotinoid insecticide with a substituted chlorothiazolyl-methyl group, which has a strong effect to control sucking and biting insects. [12]

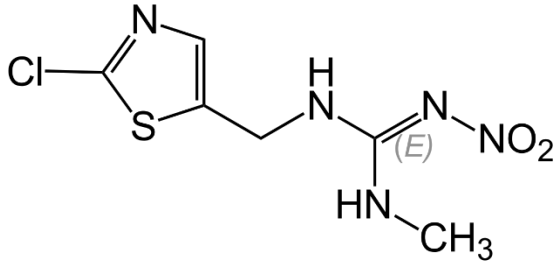

Figure 5. Structural Formula of Clothianidin

Clothianidin appears to be a persistent compound under most field conditions. Based on analysis of the laboratory studies alone, the major route of dissipation for clothianidin would appear to be photolysis if exposure to sunlight occurs. (e.g., the measured aqueous photolysis half-life was <1 day and aerobic half-lives were 148 to 1155 days). [13]

Some of the most relevant physicochemical properties of CLO for this study are summarized in this table (Table 1).

Table 2. Some relevant properties of CLO. [13]

Properties	**Values**
Molecular weight (Mw)	255.661 g/mol
Density	1.61 g/cm^3
Solubility in water	327 mg/L at 20 °C
Fusion Point	451.9 K
Photolysis Half-life	<1 day

2. OBJETIVES

The aim of this project is to study the degradation by ozonation of clothianidin, a compound of environmental concern. Concretely, the study is focused on investigate the kinetics of direct and indirect pesticide removal, which depend on hydroxyl radical (indirect way) and ozone directly (direct way), to obtain **the second order rate constants of the degradation reactions**.

In order to achieve this goal, the specific objectives were:

- Determination of the degradation kinetic constant of clothianidin photolysis under UV-C irradiation.
- Determination of the degradation kinetic constant of clothianidin by hydroxyl radicals in the UV-H_2O_2 process. The UV-H_2O_2 method was used, because the generation of hydroxyl radicals is even easier than whit ozone.
- Study the reactivity of the compound with dissolved ozone.

In addition, and because MPs are not mineralized but transformed into other chemical species, it is important to know which are these products and which risks could they pose because of their presence in the aquatic environment. Being so, preliminary studies

on the degradation mechanisms and ecotoxicity screening were also performed in this work.

3. EXPERIMENTAL SECTION

3.1 CHEMICALS AND REAGENT

First Part (kinetic study of CLO reaction with hydroxyl radical): Clothianidin (CLO, pesticide), para-Chlorobenzoic Acid (pCBA), competitor compound), Hydrogen peroxide (Reagent, 30% w/v), Phosphate buffered water.

Second Part (kinetic study of CLO reaction with ozone): Clothianidin (CLO, pesticide), Sulfamethoxazole (SMX, antibiotic competitor), Metoprolol (MET, antibiotic competitor), ozone, Tert-Butanol, Sodium Bisulfite and Indigo Reagent II (ozone scavengers), Phosphoric Acid.

The Deionized water used was treated with a purification system (Milli-Q).

3.2 ACTINOMETRY

Chemical systems for measurement of light and ultraviolet radiation are called actinometers. A chemical actinometer is a compound that undergoes a light-induced depletion process (at a certain wavelength, λ) for which the quantum yield, Φ_λ, and molar absorption coefficient, ε ($L\,mol^{-1}cm^{-1}$) is accurately known. Measuring the reaction rate allows the calculation of the absorbed photon flux, and consequently the photon flow emitted by the lamp.

3.3 DEGRADATION OF MICROPOLLUTANTS

The purpose of these experiments is to study the degradation of a micropollutant, Clothianidin, employing a UV photoreactor using a mercury lamp emitting radiation at 254 nm and ozonation by *in situ* generation ozone by electric discharge.

Initially, the experiments proposed in the following explanations were carried out:

Preparation of reagents and material:

- Prepare "stock" solution of Clothianidin, 100 mg/L (10 mg in 100 mL , water MQ) . Preparation of the calibration curve. Store in a refrigerator at ~ 4ºC.
- Prepare stock solution of phosphate buffer at pH 7, 1 M

The condition of the experiments are:

- Temperature: 22.5 ± 1 °C.
- pH=7 (phosphate Buffer Solution).

- Agitation at 600 rpm.

Phase 1: study of the CLO- • OH reaction:

- UV / H_2O_2 experiment with CLO and pCBA as a competitor, x 3.
- UV experiment with CLO and pCBA as a competitor, x 3.

Experimental conditions: Initial concentration of CLO and competitor: 1 µM (both equal); concentration initial H_2O_2: 10 ppm; Phosphate buffer concentration (pH 7): 1 mM; Irradiation time: 60 min; Sampling at: 0, 5, 10, 15, 20, 30, 45 and 60 min.

From these data it is possible to extract the following information:

i) First-order kinetic constants for the individual degradation processes (photolysis and indirect photolysis);

ii) **Second-order kinetics constants** for the reaction between clothianidin and hydroxyl radical ($k_{CLO,\bullet OH}$);

iii) Molar extinction coefficient of clothianidin at 254 nm ($\varepsilon_{CLO,254}$);

Deduction of the kinetic constant of second order by the reaction • OH-CLO from data obtained in competition kinetics experiments:

Para-chlorobenzoic acid (pCBA) was used as competitor to determine the second-order rate constants of CLO with •OH. Degradation of CLO by the UV/H_2O_2 system was due to both direct photolysis and indirect photolysis (Eq. 1). The indirect photolysis of CLO is based on its reaction with •OH:

$$-\frac{d[CLO]}{dt} = k_{obs,CLO}[CLO] = (k_{\lambda,CLO} + k_{i,CLO})[CLO] = (k_{\lambda,CLO} + k_{\cdot OH,CLO}[\cdot OH])[CLO] \quad \text{(Eq. 1)}$$

Where $k_{obs,CLO}$was the observed pseudo-first-order degradation rate constant (s^{-1}); $k_{\lambda,CLO}$ was the measured pseudo-first-order direct photolysis rate constant (s^{-1}); $k_{i,CLO}$ was the indirect photolysis rate constant (s^{-1}); and $k_{\cdot OH,CLO}$was the second-order rate constant ($M^{-1}\ s^{-1}$) of CLO with •OH, respectively.

Similarly, the degradation of pCBA can also be described using the above equation (replacing CLO by pCBA). Therefore, when CLO and pCBA were both added in the solution, the following equation, resulting from combining equations for CLO and pCBA, was obtained:

$$ln\left(\frac{[CLO]_t}{[CLO]_0}\right) - k_{\lambda,CLO} \cdot t = \left(ln\left(\frac{[pCBA]_t}{[pCBA]_0}\right) - k_{\lambda,pCBA} \cdot t\right) \cdot \frac{k_{\bullet OH,CLO}}{k_{\bullet OH,pCBA}} \quad \text{(Eq. 2)}$$

Where $k_{\lambda,CLO}$ was the measured pseudo-first-order direct photolysis rate constants (s^{-1}) and $k_{\bullet OH,CLO}$ was the second-order rate constant (M^{-1} s^{-1}) with •OH of pCBA. Equation (2) could be further expressed as below:

$$k_{i,CLO} = k_{i,pCBA} \frac{k_{\cdot OH,CLO}}{k_{\cdot OH,pCBA}} \quad \text{(Eq. 3)}$$

Where $k_{i,CLO}$ and $k_{i,pCBA}$ (the indirect photolysis rate constants (s^{-1}) of pCBA) could be obtained from the Eqs. 4 and 5:

$$k_{i,CLO} = k_{obs,CLO} - k_{\lambda,CLO} \quad \text{(Eq. 4)}$$

$$k_{i,pCBA} = k_{obs,pCBA} - k_{\lambda,pCBA} \quad \text{(Eq. 5)}$$

Applying the calculated values of $k_{i,CLO}$ and $k_{i,pCBA}$ and the known $k_{\cdot OH,pCBA}$ into the Eq. (6), the second-order rate constant of the CLO with •OH could be derived.

$$\frac{k_{obs,CLO} - k_{\lambda,CLO}}{k_{obs,pCBA} - k_{\lambda,pCBA}} = \frac{k_{i,CLO}}{k_{i,pCBA}} = \frac{k_{\bullet OH,CLO}}{k_{\bullet OH,pCBA}} \quad \text{(Eq. 6)}$$

Preparation of reagents and material:

- Prepare ozone solution; In a laboratory scrubber, made from 250 mL of water in an Erlenmeyer, the ozone flow-rate in Milli-Q water is bubbled for 30 minutes. It obtains saturation concentrations about [15-25] ppm depends on pH.

Phase 2: study of the CLO- O_3 reaction:

Competition kinetics

- Ozone experiment with CLO and SMX, fast rate.
- Ozone experiment with CLO and MET, medium rate.

The conditions of the experiments were:

- Temperature: 22.5 ± 1 °C.
- pH=7 (Buffer Solution).
- Ozone saturation at pH 7 around 18 ppm.
- Gas flow-rate and ozone concentration: (0.844 L/min , 100 g/Mm^3).

Experimental conditions: Initial concentration of CLO and competitor: 10 µM (both equal); Tert-Butanol concentration: 1 mM; Phosphate buffer concentration (pH 7): 1 mM.

We used 8 vials with different volumes of ozone sample: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 mL, all of them filled at 20 mL of the sample with CLO and the competitor and buffered water up to 25 mL.

Direct kinetic study

- Ozone experiment with CLO, degradation as function of time.

The conditions of the experiments were:

- Temperature: 22.5 ± 1 °C.
- PH=7 (Buffer Solution).
- Ozone saturation at pH 2 around 22.5 ppm.
- Gas flow-rate and ozone concentration: (0.844 L/min, 100 g/Nm^3).

Experimental conditions: Initial concentration of CLO: 1 ppm; Tert-Butanol concentration: 1 mM; Bisulfite: 10 µL, Acid Solution (H_2PO_4, pH ≈ 2).

At Stirred reactor of 275 mL, 1/8 of the ozone sample and 7/8 of the sample with the micropollutant are introduced, sampling every 30 second until 5 minutes and them, the last one at 10 minutes.

From these data, it is possible to extract the following information:

i) **Second-order kinetics constants** for the reaction between clothianidin and Ozone ($k_{CLO\ ,\ O3}$);

ii) The Ozone Concentration at time (Indigo Colorimetric method);

Deduction of the kinetic constant of second order by the reaction O_3-CLO from data obtained in degradation kinetics experiments:

It is similar than the kinetic degradation with hydroxyl radical, because the ozone has two ways, one with • OH and the other one with O_3.

$$\frac{-d[CLO]}{dt} = k_{O3,CLO}.[O_3].[CLO] + k_{\bullet OH,CLO}.[\bullet OH].[CLO] \quad \text{(Eq. 7)}$$

In this case, the hydroxyl radical of the reaction, which are produced by ozone, are eliminated by the action of tert-Butanol. Hence, we can remove the second term.

Therefore, if we control the time and the concentration of ozone, it is possible obtain the second order rate constant by ozone ($k_{O3,CLO}$).

$$-\ln\left(\frac{CLO}{CLO_o}\right) = k_{O3,CLO} * [O_3] * t \quad \text{(Eq. 8)}$$

3.4 ECOTOXYCITY (*Vibrio Fischeri*)

Vibrio fischeri bioluminescence inhibition bioassay (VFBIA) has been widely applied for the monitoring of toxicity on account of multiple advantages encompassing shorter test duration, sensitive, cost-effective and ease of operation. Moreover, this bioassay found to be equally applicable to all types of matrices (organic & inorganic compounds, metals, wastewater, river water, sewage sludge, landfill leachate, herbicides, treated wastewater etc.) for toxicity monitoring.[14]

3.5 ANALYTICAL METHODS

High-performance liquid chromatography (HPLC)

It is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.

Column: *Teknokroma Mediterranea Sea* C18, 250x4.6 mm, 5 µm packaging
Flow-rate: 1 mL/min, Mobile phase: 50% acetonitrile, 50% Milli-Q water at PH 3,
Detection wavelength: 236 nm (pCBA), 268 nm (CLO).

With the aim of elucidating the ACMP degradation pathways given in ozonation process, samples in which different ozone doses were applied were analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS). An Agilent 1100 HPLC coupled with a G1969A LC/MSD-TOF mass spectrometer was employed. MS data were collected in full scan mode (25 – 1000 m/z), employing positive electrospray ionization.

Indigo colorimetric method (Standard Methods 4500-O_3)

The indigo colorimetric method is quantitative, selective, and simple; it replaces methods based on the measurement of total oxidant. The method is applicable to lake water, river infiltrate, manganese-containing groundwaters, extremely hard groundwaters, and even biologically treated domestic wastewaters.

In acidic solution, ozone rapidly decolorizes indigo. The decrease in absorbance is linear with increasing concentration. The proportionality constant at 600 nm is 0.42 ± 0.01/cm/mg/L (Δ = 20 000/M·cm) compared to the ultraviolet absorption of pure ozone of = 2950/M·cm at 258 nm).

Indigo stock solution: Add about 500 mL distilled water and 1 mL conc phosphoric acid to a 1-L volumetric flask. With stirring, add 770 mg potassium indigo trisulfonate, $C_{16}H_7N_2O_{11}S_3K_3$ (use only high-grade reagent, commercially available at about 80 to 85 % purity). Fill to mark with distilled water

Indigo reagent II: To a 1-L volumetric flask add 100 mL indigo stock solution, 10 g sodium dihydrogen phosphate (NaH_2PO_4), and 7 mL conc phosphoric acid. Dilute to mark. [15]

Calculation:

$$mg\ O_3/L\ = \frac{100 * \Delta A}{f * b * V} \qquad \text{(Eq. 9)}$$

Where:

ΔA = difference in absorbance between sample and blank,

b = path length of cell, cm,

V = volume of sample, mL (normally 90 mL), and

f = 0.42.

Ultraviolet spectroscopy

Ultraviolet spectroscopy is a technic used in chemical analysis used to measure, as a function of wavelength, the relationship between values of the same photometric magnitude relative to two radiation beams and the concentration or chemical reactions that are measured in a sample. It is also used in chemistry laboratories for the quantification of substances and microorganisms.

Spectrophotometer HACH, UV-Vis DR model 6000.

Detection wavelength: 260 nm (Ozone), 600 nm (Azul Indigo II).

4. RESULTS

4.1 Absorption spectra and molar extinction

At first, it was necessary characterize the compound and its absorption spectrum. Therefore, it a UV-Vis scan was realized to determine the absorption spectra

and considerate if the lamp that will use in the laboratory will be a suitable option for the degradation of this chemical.

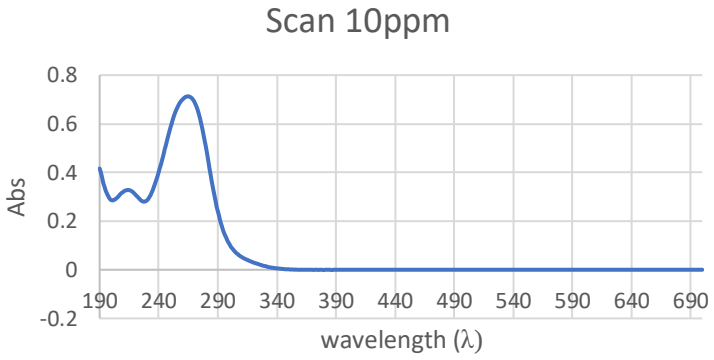

Figure 6. Absorption spectra of Clothianidin

From the lambert-beer equation, the molar extinction coefficient can be obtained.

$$Abs = \varepsilon * c * l \quad \text{(Eq. 10)}$$

Table 3. Molar Extinction Coefficient at 254nm

$\varepsilon_{CLO,254}$	0.064	L/(mg.cm)
$\varepsilon_{CLO,254}$	15979.392	L/(mol.cm)

As a result of the study, the clothianidin has a peak of absorption at 265nm and the low-pressure mercury lamp can be used to degrade the insecticide.

4.2 Study of removal of CLO by indirect degradation (photolysis and [·OH])

It is necessary to do a calibration to obtain a correct measure in the HPLC. Calibration curve was realized with different concentration of our chemical compound in Milli-Q water (2, 1, 0.5, 0.25, 0.125ppm and 0ppm), and samples were introduced in the analyzer.

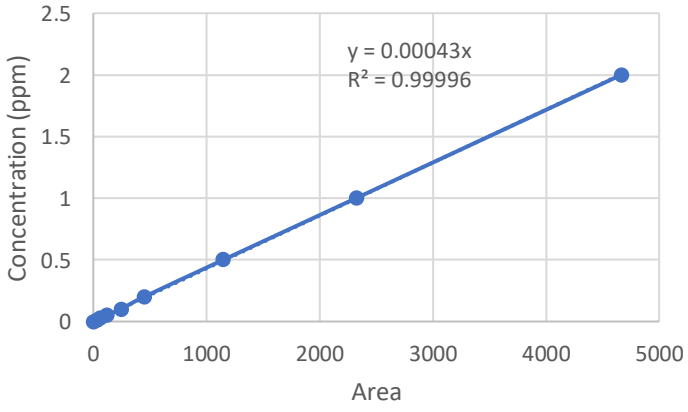

Figure 7. Calibration Curve

To start with the indirect degradation, initial sample, which include the micropollutant and the competitor, is introduced in the reactor and applied only UV at 254nm to observe the removal of the contaminant at 60 minutes. And the same processes for UV-H_2O_2.

Figure 8 and 9 show the result of the experiments previously described.

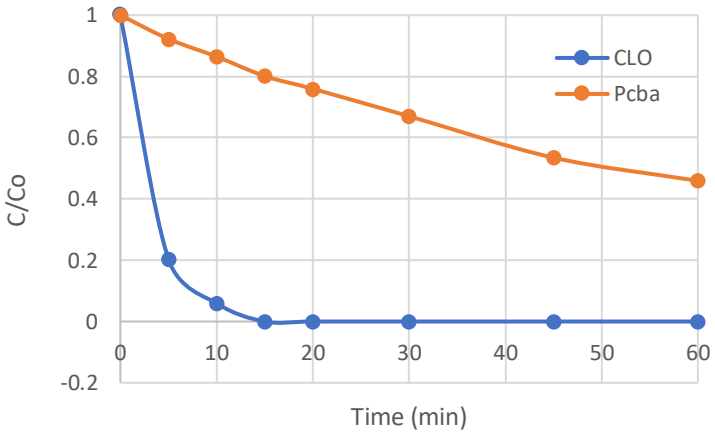

Figure 9. First experiment UV, 60'

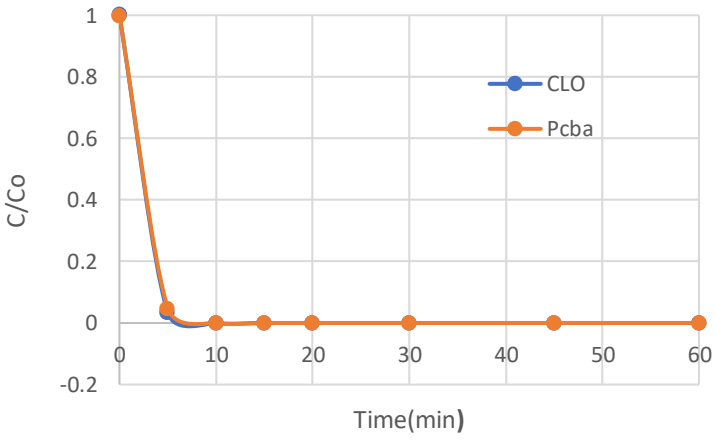

Figure 8. First experiment UV-H_2O_2, 60'

The experiment shows that Clothianidin has a fast rate of decomposition with UV light, but it is not the same for pCBA, due to the removal is slowly only with irradiation.

On the other hand, with UV-H_2O_2 both compounds suffer a great decomposition in a few minutes, which is not convenient for the purposes of the experiments. Consequently, the new experiments will be in a short time, around 10 minutes, to observe the degradation in deep.

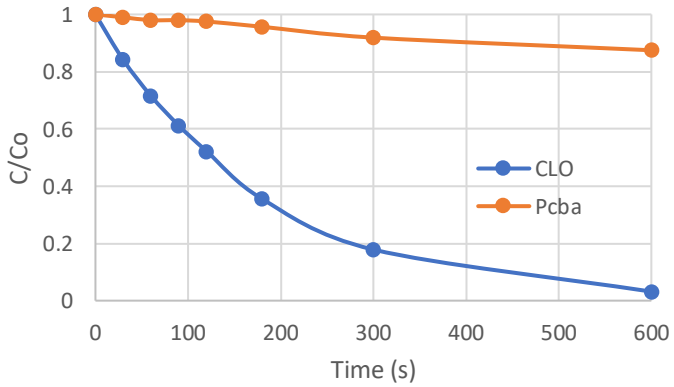

Figure 11. Experiment UV, 10'

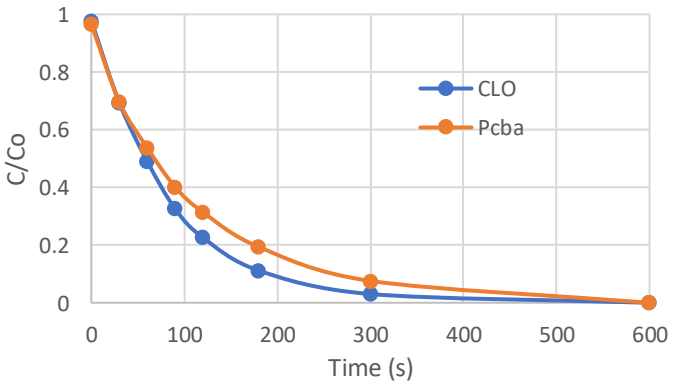

Figure 10. Experiment UV-H_2O_2, 10'

To continue, some experiments were performed per triplicate in order to obtain the first-order kinetic constants of the different processes involved (that is, photolysis and photolysis combined with hydroxyl radical degradation) ($k_{\lambda,CLO}, k_{obs,CLO}$).

These constants are calculated with the natural logarithm of final concentration per initial concentration represented as a function of time.

$$-\frac{d[CLO]}{dt} = k_{\lambda,CLO} * [CLO] \xrightarrow{\text{Integrated}} -\ln(\frac{CLO}{CLO_o}) = k_{\lambda,CLO} * t \quad \text{(Eq. 11)}$$

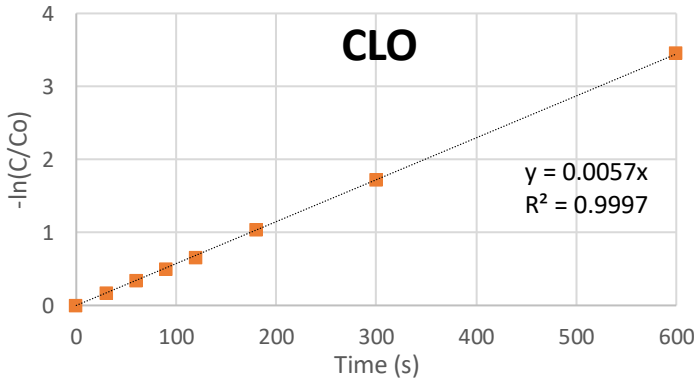

Figure 12. Linear Regression of CLO by UV

This equation is for the degradation with UV light and we get direct constant. If UV-H_2O_2 is used, observed kinetic constant will be got which depend on [·OH].

$$-\frac{d[CLO]}{dt} = k_{d,CLO} * [CLO] + k_{i,CLO} * [CLO] \longrightarrow -\frac{d[CLO]}{dt} = (k_{\lambda,CLO} + k_{i,CLO}) * [CLO]$$

$$-\frac{d[CLO]}{dt} = k_{obs,CLO} * [CLO] \xrightarrow{\text{Integrated}} -\ln(\frac{CLO}{CLO_o}) = k_{obs,CLO} * t \quad \text{(Eq. 12)}$$

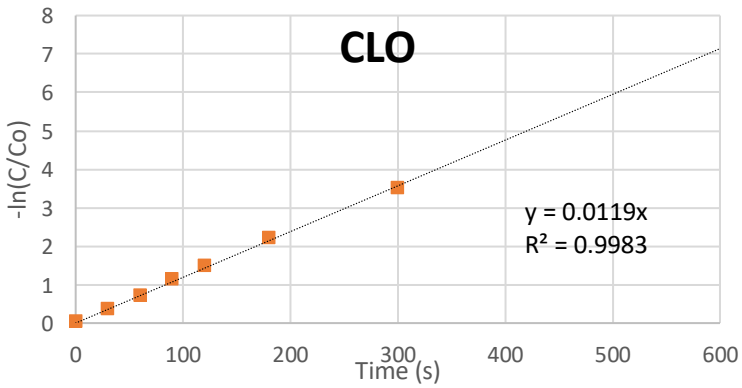

Figure 13. Linear Regression of CLO by UV-H_2O_2

Table 4. Observed Kinetic Constant (UV-H2O2)

	CLO	pCBA
Kobs1	0.0130	0.0105
Kobs2	0.0119	0.0089
Kobs3	0.0117	0.0080
Media	0.0118	0.0084
standard deviation	1.34E-04	6.65E-04

Table 5. Direct Kinetic Constant (UV)

	CLO	pCBA
Kλ1	0.0059	0.00036
Kλ2	0.0057	0.00023
Kλ3	0.0056	0.00017
Media	0.0057	0.00025
standard deviation	1.75E-04	9.71E-05

The values in tables 4 and 5 correspond to the experimental pseudo-first order rate constants. However, the aim of this thesis is obtaining the second-order rate constant which is calculated by eq. 2. This equation involves indirect constant to obtain "$k_{.OH,CLO}$".

The secondary kinetic constant for pCBA is: $k_{.OH,Pcba} = 5 * 10^9 M^{-1}s^{-1}$ [16]

Table 6. Indirect Kinetic Constant

	CLO	pCBA
Ki	0.006018333	0.00823667
standard deviation	0.000309088	0.00076181

Table 7. Second-Order Kinetic Constant for CLO reaction with hydroxyl radical.

$K_{OH,\,CLO}$	3.6534E+09	$M^{-1}.s^{-1}$
standard deviation	0.1438E+09	

It was obtained that $k_{.OH,CLO} = 3.65 * 10^9 \pm 0.14 * 10^9\ M^{-1}s^{-1}$

4.3 Study of removal of CLO by direct ozone degradation ([O_3])

In this second study, we used two different experiments to determinate the second order rate constant of CLO degradation by O_3: Kinetic Competition and Degradation as function of time.

Firstly, it is necessary a calibration method for the analyses of the new compounds, Sulfamethoxazole (SMX) and Metoprolol (MET), for its correct measurement by HPLC.

Kinetic Competition

At first, a kinetic competition was done to get an idea about the reactivity of ozone with Clothianidin.

Two micropollutants with different ozone reactivity were used as competitors.

High Reactivity (Sulfamethoxazole ≈ 10^6 M^{-1} s^{-1}), Kinetic Competition.

In first place, it was assumed that CLO readily reacted with ozone. Being so, the antibiotic SMX, a compound reacting with second-order kinetics of about 10^6 $M^{-1}s^{-1}$ was selected as competitor.

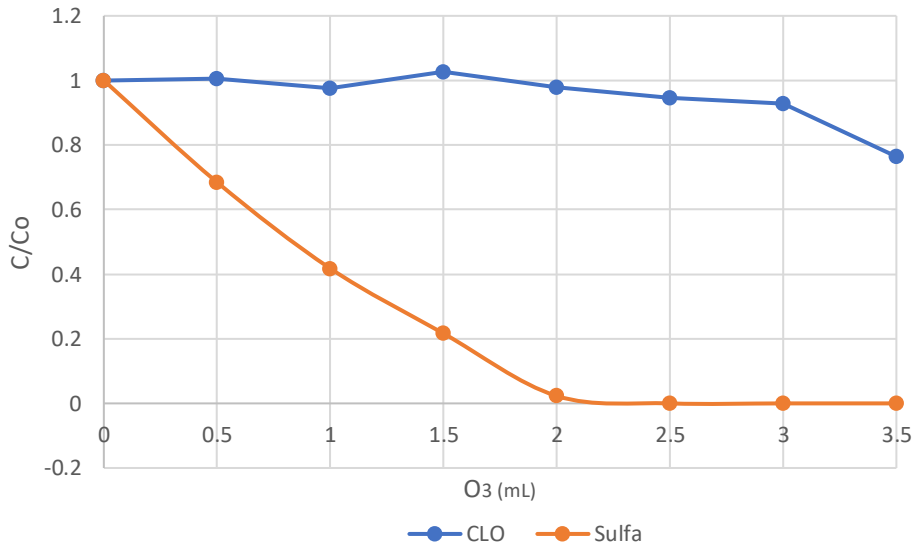

Figure 14. Degradation Curve (Sulfa and CLO)

Figure 14 displays the decomposition of Clothianidin and Sulfamethoxazole as function of different ozone concentrations, it can observe that SMX is much more reactive than CLO. The competition is not possible due to the fact that SMX scavenges all the ozone. In fact, it can be observed that whereas SMX was not degraded, CLO did not start its removal.

Sulfamethoxazole is much faster than CLO regarding its degradation by ozone, so it could not be used as competitor in these experiments

Moderate Reactivity (Metoprolol ≈ 10^4 M^{-1} s^{-1}), Kinetic competition.

In a similar way than with SMX, the kinetic competition was also studied with MET, another micropollutant which is slower than SMX regarding its reaction with ozone (second-order rate constant about 10^3 $M^{-1}s^{-1}$).

The result of this experiment is shown in Figure 15, as the first experiment, the new competitor is also much faster than CLO and the comparison is again not possible.

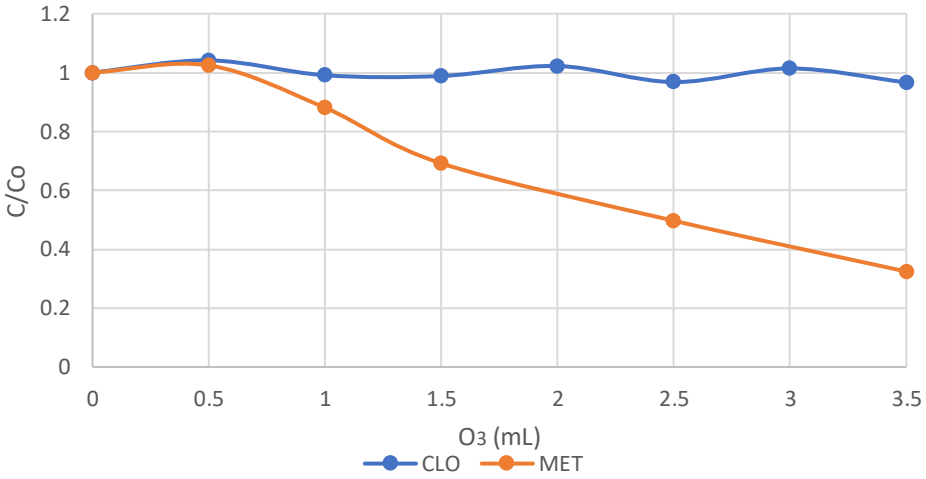

Figure 15. Degradation Curve (MET and CLO).

As previously advanced, a second type of experiment was done because an appropriate competitor was not found and also because CLO, according to the previous experiments, appeared to react slow with ozone, being thus possible to follow its degradation by a direct method rather than by an indirect experiment such as competition kinetics. The new method consisted of monitoring the degradation of CLO over time. To obtain the second order rate constant by means of this methodology, however, it was necessary to control both CLO and Ozone Concentration.

The Ozone concentration measure by Indigo colorimetric method, which is explained at point 3.5.

Kinetic Degradation

As a result of study, Clothianidin is practically degraded after 5 minutes of experiment. The Ozone concentration for these experiments at the moment its introduce at stirred reactor are 2.5 ppm.

Table 8. Ozone Concentration.

Replicate 1, [O_3]	
2.405	mg/L
5.010E-05	mol/L

Replicate 2, [O_3]	
2.328	mg/L
4.851E-05	mol/L

Table 8 shows the ozone concentration. As experiments were performed under acidic conditions with excess of ozone with respect to CLO, the dissolved ozone is stable and therefore its concentration can be considered constant. Table 9 represents measurements of ozone concentration as a function of time during degradation experiments

Table 9. Ozone Concentration as function of Time.

Time (Seconds)	[O_3] mg/L
30	2.314
60	2.443
90	2.329
120	2.471
150	2.471
180	2.386
210	2.400
240	2.657
270	2.429
300	2.086
600	2.471

Figure 15 shows CLO decomposition by the action of Ozone. As it can be seen, all the compound was removed in approximately 5 minutes. The shorter time of degradation depended on the ozone concentration that has been introduced in the sample, in our case 2.5 ppm. If The ozone concentration is higher, the rate of decomposition will probably increase.

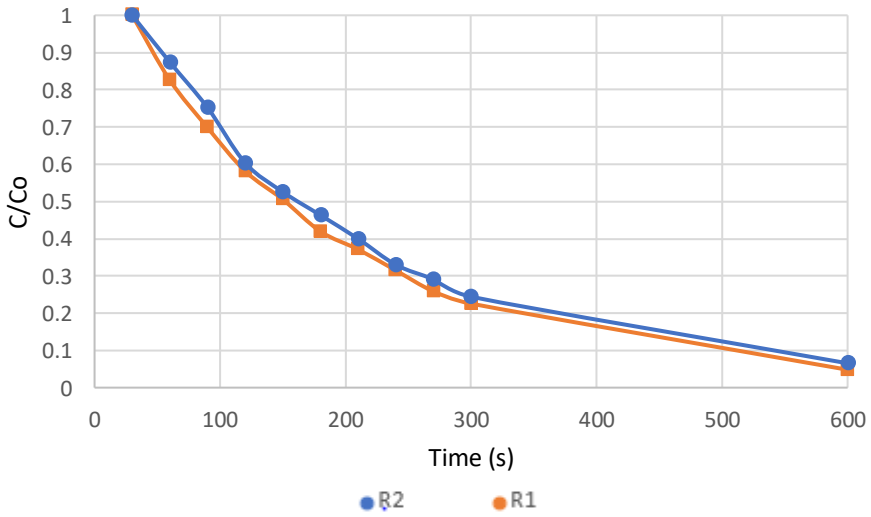

Figure 16. Clothianidin decomposition as function of Time

To calculate the Second order constant of CLO by ozone, the following equation was used:

$$-\frac{d[CLO]}{dt} = k_{obs\ O3,CLO} * [CLO] \longrightarrow -\frac{d[CLO]}{dt} = k_{O3,CLO} * [O_3] * [CLO] \quad \text{(Eq. 13)}$$

As the ozone concentration remains constant over the time (see O_3 concentration data in Table 9). Hence, the equation to obtain the linear regression and the second order constant ($k_{O3,CLO}$) is calculated.

$$-\frac{d[CLO]}{dt} = k_{O3,CLO} * [O_3] * [CLO] \xrightarrow{\text{Integrated}} -\ln\left(\frac{CLO}{CLO_o}\right) = k_{O3,CLO} * [O_3] * t \quad \text{(Eq. 14)}$$

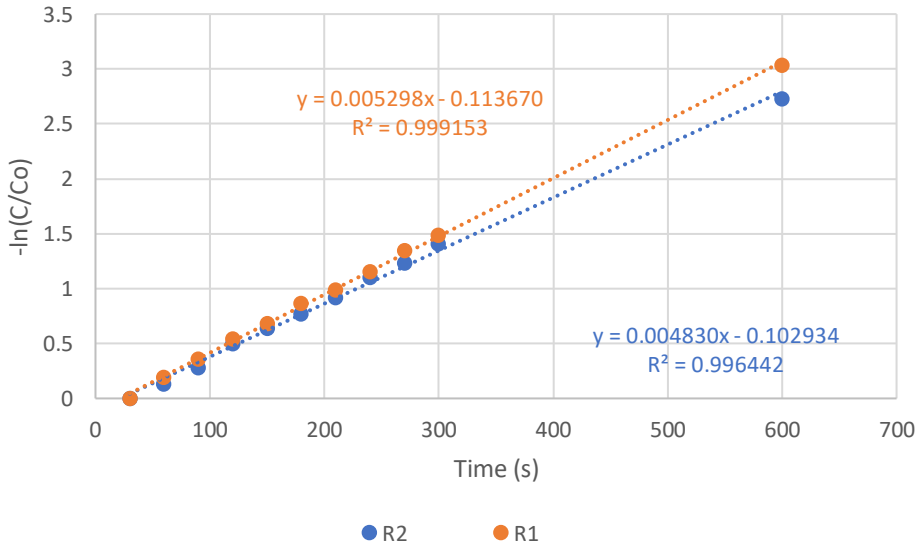

Figure 17. Linear regression of CLO by Ozone

Table 10. Second order constant, First experiment

CLO, R1		
$K_{ObsO3,\ CLO}$	0.005298	s^{-1}
$K_{O3,\ CLO}$	105.732953	$M^{-1}s^{-1}$

Table 11. Second order constant, Second experiment

CLO, R2		
$K_{ObsO3,\ CLO}$	0.004830	s^{-1}
$K_{O3,\ CLO}$	99.562184	$M^{-1}s^{-1}$

Finally, the second order rate constant of CLO by Ozone is:

$$k_{O3,CLO} = 102.64 \pm 4.36\ M^{-1}s^{-1}$$

4.4 Study of Clothianidin *Ecotoxicity*

Two samples with different concentrations, 1ppm and 0,5 ppm, were prepared for these experiments.

The ecotoxicity show us, The **Half maximal effective concentration** (EC_{50}), which is the concentration of a toxic compound that produces an effect (in the case of Vibrio fischeri the inhibition of its natural luminescence) in 50% of the population to which you expose the toxin during a given time. In the experiment the time response is 15 minutes.

Table 12. Ecotoxicity at 1ppm of CLO

Bucket		Initial reading	15 minutes
b1		92	84
	Sample Concentration	Average proportion of targets	0,9130
b2	*0.13*	98	89
b3	*0.25*	77	70
b4	*0.50*	102	90
b5	*1.00*	92	83

RESULTADO:

EC_{50}	428.08	mol/L

Table 13. Ecotoxicity at 0,5ppm of CLO

Bucket		Initial reading	15 minutes
b1		94	92
	Sample Concentration	Average proportion of targets	0,9787
b2	*0.06*	81	79
b3	*0.13*	89	87
b4	*0.25*	94	91
b5	*0.50*	69	67

RESULTADO:

CE_{50}	574.63	mol/L

As we can see, the ecotoxicity of this compound is really low, after 15minutes the concentration of Vibrio fischeri just decrease 3-4 pints respect to the initial

concentration. It was decided to do another experiment with a lower concentration and the result was similar.

For this, the concentration to kill 50% of bacterial population is around 500 mol/L. It could be said that ecotoxicity exerted by CLO to this type of bacteria is low, although this situation could be different for other living organisms.

4.5 Degradation Pathways

Because of the fact that Hydroxyl Radical and Ozone are powerful oxidants, CLO is degraded, but in the removal process this compound is not completely mineralized and some transformation products are formed.

First of all, the figure 17 represent the Clothianidin and the pathways begin from it.

One of these **preliminary transformation products** is that appears in the following;

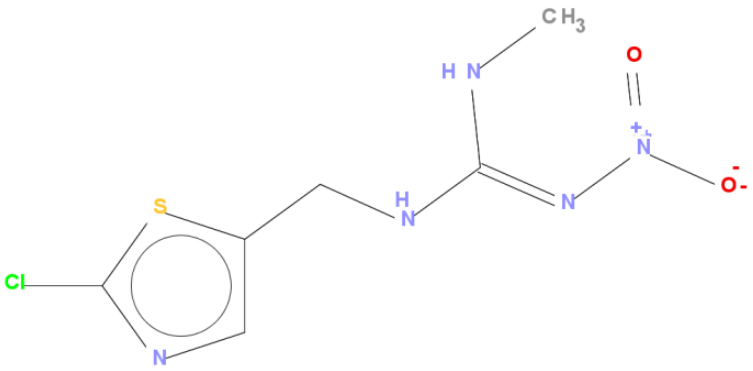

Figure 18. Clothianidin Structure

Table 14. Transformation Products

Compound	Formula	Experimental mass *(m/z)*
CLO	$C_6H_8N_5O_2SCl$	250
TP1	$C_5H_6N_5O_2SCl$	235
TP2	$C_4H_8N_4O_4$	176
TP3	$C_2H_6N_4O_2$	118

Transformation product 1 (TP1) *m/z* = 235

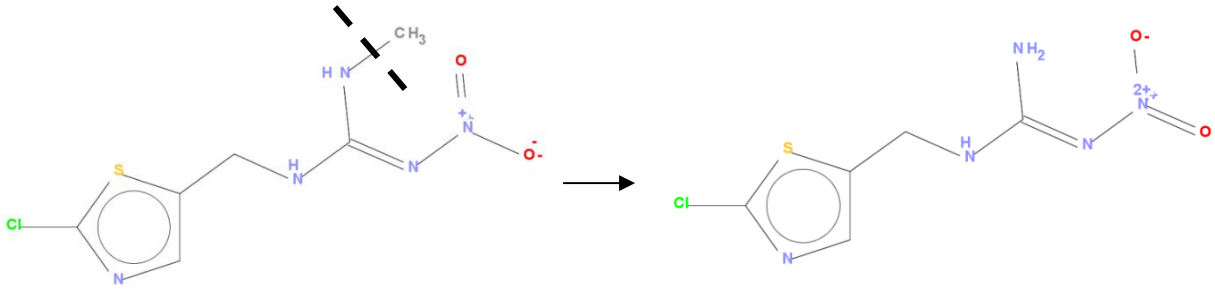

Figure 19. TP1 Structure

TP1 is the first product and its molecular weight is 235 versus 250 of CLO. It was formed by the lost of methyl group and create this new product.

To know the ecotoxicity of this sub-product, we use The Ecological Structure Activity Relationships (ECOSAR), is a computerized predictive system that estimates aquatic toxicity. For TP1 Table 15 represents the ecotoxicity of the compound for different living organism, in this table it is necessary to highlight the toxicity produced to the daphnia, which with small concentrations already suffer great damage.

Table 15. TP1 Ecotoxicity

Nicotinoids			
Organism	**Duration**	**End Point**	**Concentration (mg/L)**
Fish	96h	LC50	71.2
Daphnid	48h	LC50	0.293
Green Algae	96h	EC50	6.74E+3

Transformation Product 2 (TP2), *m/z* = 176

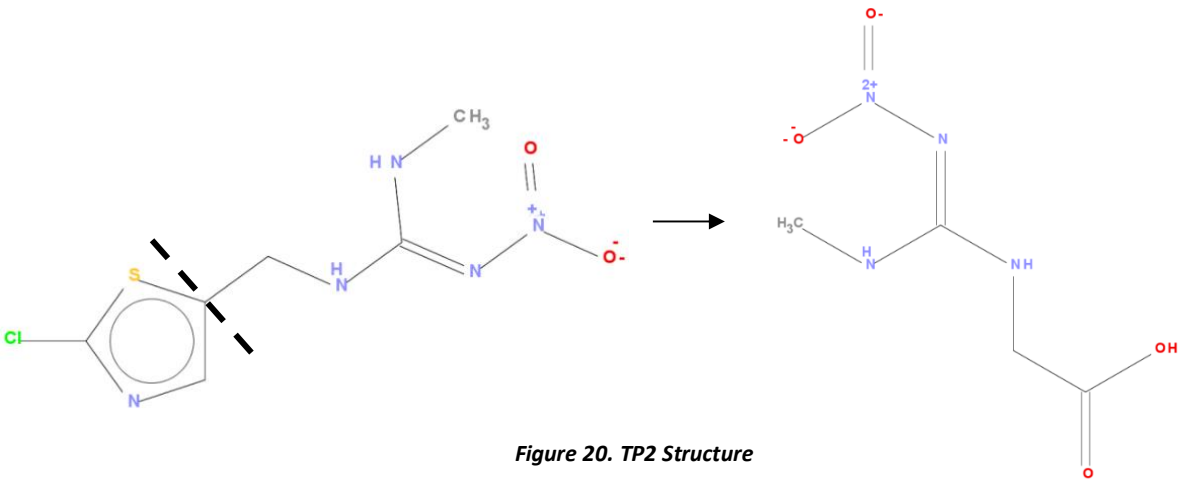

Figure 20. TP2 Structure

TP2 m/z 176 was formed by the lost of aromatic ring and a carboxyl group was added, It was assigned to molecular formula $C_4H_8N_4O_4$.

About the ecotoxicity, Ecosar show us zero toxicity for this compound, higher concentration could make a little damage at the living organism.

Table 16. TP2 Ecotoxicity

Organism	Duration	End Point	Concentration (mg/L)
Fish	96h	LC50	4.89E+7
Daphnid	48h	LC50	2.34E+6
Green Algae	96h	EC50	1.17E+7

Transformation Product 3 (TP3), *m/z* = 118

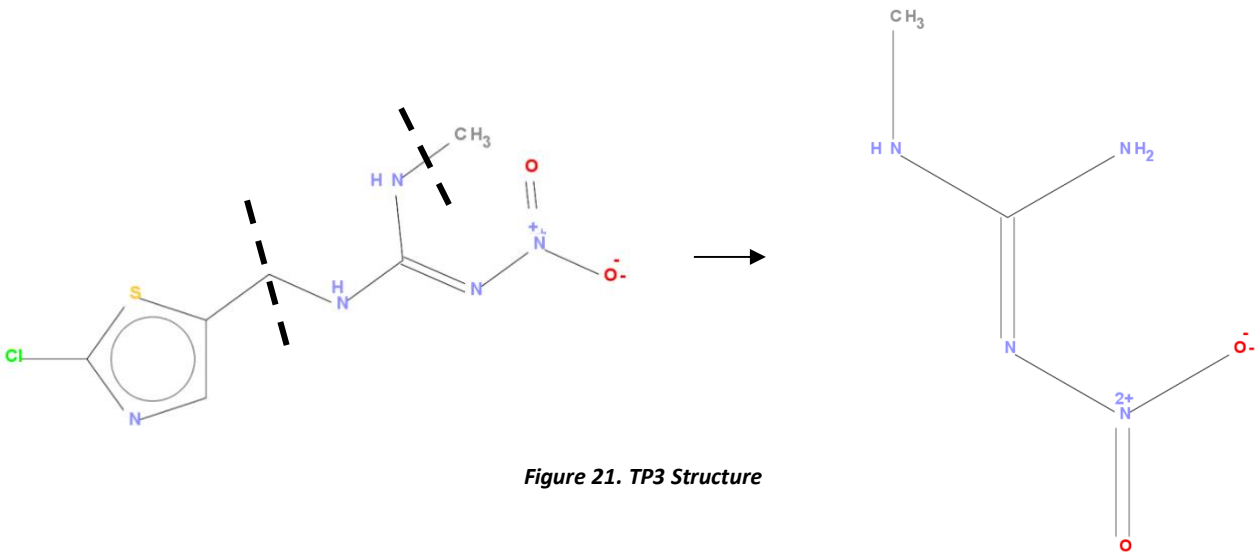

Figure 21. TP3 Structure

This transformations product could be one product of TP1. TP3 was formed by the lost of aromatic ring and methyl group. This compound has very low toxicity and it has not really importance from aqueous matrices.

Table 17.TP3 Ecotoxicity

Organism	Duration	End Point	Concentration (mg/L)
Fish	96h	LC50	1.01E+5
Daphnid	48h	LC50	6.30E+3
Green Algae	96h	EC50	1.86E+4

5 CONCLUSION

Taking into account the development of the main objective of this master thesis, it has been possible to conclude:

- The clothianidin (CLO) is a very photosensitive compound at 254 nm, however, the pCBA remains stable using UV light.
- It has observed UV-H_2O_2 degradation combining the effect of light and hydroxyl radicals and obtaining Kobs very similar for pCBA and CLO.
- Compared to other compounds, the CLO reacts much slower, although it can not be considered a recalcitrant compound to ozone since in fact if it is eliminated.
- The second order kinetic constant of CLO degradation with [•OH], obtained in this study, is $k_{\bullet OH/CLO} = 3.65.10^9 \pm 0.14\ M^{-1}s^{-1}$. This means that, according to specific bibliography the reactivity of CLO with the hydroxyl radical is moderate. [17]
- The second order kinetic constant of clothianidin with [O_3] is $k_{O3,CLO} = 102.64 \pm 4.36\ M^{-1}s^{-1}$. According to this value, this micropollutant can be classified as a compound with moderate reactivity with ozone, between 10-10^5. [17]
- The ecotoxicity of this microcontaminant for the vibro fischeri microtox is negligible. However, these results are not conclusive enough and its toxicity should be tested for other living organisms, since this is a pesticide of new generation designed for target toxicity to insects.
- The preliminary transformation products that have been obtained, TP1, TP2 and TP3 do not present apparent toxicity according to ECOSAR estimations. For future work, it would be interesting to make depth study of these degradation pathways and the associated toxicity of formed TPs.

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